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Vascular effects and safety of dalcetrapib in patients with or at risk of coronary heart disease: the dal-VESSEL randomized clinical trial

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Abstract: Aims High-density lipoprotein cholesterol (HDL-C) is inversely associated with cardiovascular (CV) events and thus an attractive therapeutic target. However, in spite of marked elevations in HDL-C, the first cholesterol transport protein (CETP) inhibitor torcetrapib raised blood pressure (BP), impaired endothelial function, and increased CV mortality and morbidity. Dalcetrapib is a novel molecule acting on CETP with a different chemical structure to torcetrapib. As HDL stimulates nitric oxide (NO), suppresses inflammation, and exerts protective CV effects, we investigated the effects of dalcetrapib on endothelial function, blood pressure, inflammatory markers, and lipids in patients with, or at risk of, coronary heart disease (CHD) in a double-blind randomized placebo-controlled trial (clinicaltrials.gov number NCT00655538). Methods and results Patients with target low-density lipoprotein cholesterol (LDL-C) levels received dalcetrapib 600 mg/day or placebo for 36 weeks on top of standard therapy (including statins). The primary outcome measures were the change from baseline of flow-mediated dilatation (%FMD) of the right brachial artery after 5 min of cuff occlusion at 12 weeks and the 24 h ambulatory blood pressure monitoring (ABPM) at week 4. Secondary outcomes included change from baseline in FMD after 36 weeks and the change in ABPM at 12 and 36 weeks, changes in HDL-C, LDL-C, triglycerides, CETP activity, as well as standard safety parameters. Four hundred seventy-six patients were randomized. Baseline FMD was 4.1 ± 2.2 and 4.0 ± 2.4% with placebo or dalcetrapib, respectively and did not change significantly from placebo after 12 and 36 weeks (P = 0.1764 and 0.9515, respectively). After 4, 24, and 36 weeks of treatment with dalcetrapib, CETP activity decreased by 51, 53, and 56% (placebo corrected, all P < 0.0001), while at weeks 4, 12, and 36 HDL-C increased by 25, 27, and 31% (placebo corrected, all P < 0.0001). Low-density lipoprotein cholesterol levels did not change. At baseline, ABPM was 125 ± 12/74 ± 8mmHg in the placebo and 128 ± 11/75 ± 7mmHg in the dalcetrapib group (P = 0.3372 and 0.1248, respectively, placebo-corrected change from baseline) and did not change for up to 36 weeks. Biomarkers of inflammation, oxidative stress, and coagulation did not change during follow-up except for Lp-PLA2 mass levels which increased by 17% (placebo corrected). Overall 7 patients given dalcetrapib and 8 patients given placebo experienced at least one pre-specified adjudicated event (11 events with dalcetrapib and 12 events with placebo). Conclusion The dal-VESSEL trial has established the tolerability and safety of CETP-inhibition with dalcetrapib in patients with or at risk of CHD. Dalcetrapib reduced CETP activity and increased HDL-C levels without affecting NO-dependent endothelial function, blood pressure, or markers of inflammation and oxidative stress. The dal-OUTCOMES trial (NCT00658515) will show whether dalcetrapib improves outcomes in spite of a lack of effect on endothelial function

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Vascular effects and safety of dalcetrapib in patients with or at risk of coronary heart disease: the dal-VESSEL randomized clinical trial

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Aims High-density lipoprotein cholesterol (HDL-C) is inversely associated with cardiovascular (CV) events and thus an attractive therapeutic target. However, in spite of marked elevations in HDL-C, the first cholesterol transport protein (CETP) inhibitor torcetrapib raised blood pressure (BP), impaired endothelial function, and increased CV mortality and morbidity. Dalcetrapib is a novel molecule acting on CETP with a different chemical structure to torcetrapib. As HDL stimulates nitric oxide (NO), suppresses inflammation, and exerts protective CV effects, we investigated the effects of dalcetrapib on endothelial function, blood pressure, inflammatory markers, and lipids in patients with, or at risk of, coronary heart disease (CHD) in a double-blind randomized placebo-controlled trial (clinicaltrials.gov number NCT00655538).

Methods and results Patients with target low-density lipoprotein cholesterol (LDL-C) levels received dalcetrapib 600 mg/day or placebo for 36 weeks on top of standard therapy (including statins). The primary outcome measures were the change from baseline of flow-mediated dilatation (%FMD) of the right brachial artery after 5 min of cuff occlusion at 12 weeks and the 24 h ambulatory blood pressure monitoring (ABPM) at week 4. Secondary outcomes included change from baseline in FMD after 36 weeks and the change in ABPM at 12 and 36 weeks, changes in HDL-C, LDL-C, triglycerides, CETP activity, as well as standard safety parameters. Four hundred seventy-six patients were randomized. Baseline FMD was 4.1 $\pm$ 2.2 and 4.0 $\pm$ 2.4% with placebo or dalcetrapib, respectively and did not change significantly from placebo after 12 and 36 weeks ($P = 0.1764$ and 0.9515, respectively). After 4, 24, and 36 weeks of treatment with dalcetrapib, CETP activity decreased by 51, 53, and 56% (placebo corrected, all $P < 0.0001$), while at weeks 4, 12, and 36 HDL-C increased by 25, 27, and 31% (placebo corrected, all $P < 0.0001$). Low-density lipoprotein cholesterol levels did not change. At baseline, ABPM was 125 $\pm$ 12/74 $\pm$ 8 mmHg in the placebo and 128 $\pm$ 11/75 $\pm$ 7 mmHg in the dalcetrapib group ($P = 0.3372$ and 0.1248, respectively, placebo-corrected change from baseline) and did not change for up to 36 weeks. Biomarkers of inflammation, oxidative stress, and coagulation did not change during follow-up except for Lp-PLA_2 mass levels which increased by 17% (placebo corrected). Overall 7 patients given dalcetrapib and 8 patients given placebo experienced at least one pre-specified adjudicated event (11 events with dalcetrapib and 12 events with placebo).

Conclusion The dal-VESSEL trial has established the tolerability and safety of CETP-inhibition with dalcetrapib in patients with or at risk of CHD. Dalcetrapib reduced CETP activity and increased HDL-C levels without affecting NO-dependent endothelial function, blood pressure, or markers of inflammation and oxidative stress. The dal-OUTCOMES trial (NCT00658515) will show whether dalcetrapib improves outcomes in spite of a lack of effect on endothelial function.

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Keywords High-density • Lipoprotein • Cholesterol (HDL-C) • Torcetrapib • Dalcetrapib

Introduction

Lowering of low-density lipoprotein cholesterol (LDL-C) by inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (i.e. statins) is highly effective in improving cardiovascular (CV) outcome in a broad range of patients.[1–3] However, considerable residual risk remains, particularly in patients with low high-density lipoprotein cholesterol (HDL-C).[4] Epidemiological and experimental data support a protective role for HDL-C against atherosclerotic vascular disease. Thus, drugs which increase HDL-C would provide a therapeutic opportunity to reduce further CV events further.

Targeting the activity of the cholesteryl ester transfer protein (CETP), a plasma protein that promotes transfer of cholesteryl esters from protective HDL-C to atherogenic LDL particles, is a promising approach to increase HDL-C.[5] The first CETP inhibitor, torcetrapib,[6] substantially increased HDL-C, but unexpectedly led to an increased mortality in a large outcome trial.[7] Subsequent studies showed that torcetrapib has a number of 'off-target' effects, including increases in blood pressure (BP), in the release of aldosterone and endothelin, and—in experimental models—marked endothelial dysfunction as well as worsening of angina.[8–11] These findings were surprising as HDL-C increases endothelial nitric oxide (NO) synthase expression,[12–15] NO release, and bioavailability (due to its superoxide scavenging properties),[16] and augment flow-mediated dilatation (FMD) in patients with hypercholesterolaemia.[17] As the untoward effects of torcetrapib were also observed in animals lacking CETP,[9,11,18] they are unlikely to be related to its action on CETP. Nevertheless, demonstration of the absence of such off-target toxicity has become a prerequisite for the development of novel CETP inhibitors.

Dalcetrapib is one of several molecules affecting CETP activity[19,20] that increases HDL-C, apparently without the side effects of torcetrapib. However, both its CV safety and efficacy remain uncertain. As the vascular protective effects of HDL-C are mediated in part by activation of endothelial cells, in particular the release of NO[16] and as torcetrapib specifically worsened this response, in the dal-VESSEL trial[21] we chose to use endothelial function as assessed by FMD as the primary outcome measure. We enrolled patients with average or low HDL-C with or at risk of coronary heart disease (CHD) to exclude 'torcetrapib-like' effects in a non-inferiority trial.

Methods

Study design

The concept and design of the dal-VESSEL trial (see clinicaltrials.gov number NCT00655538) have been published.[21] The study protocol was approved by the institutional review boards or ethical committees from participating institutions, respectively. Informed consent was obtained from all patients before entering the trial. Eligible patients with CHD or CHD risk equivalent and HDL-C <50 mg/dL were required to be treated with statins or ezetimibe to a LDL-C level <100 mg/dL. Four hundred seventy-six patients were randomized 1:1 between June 2008 and August 2009 to either placebo or dalcetrapib 600 mg/day in a double-blind fashion. The dalcetrapib dose (600 mg/day) was based on previous studies.[19,22,23] Patients were instructed to take two tablets of 300 mg or placebo daily, respectively, with a meal.

Randomization and masking

Patients were assigned to one of two treatment groups and stratified by centre according to a computer-generated global randomization code. Blinding was maintained through matching placebo and dalcetrapib tablets, and with regard to participant HDL-C and total cholesterol levels.

Concomitant treatment

Patients were advised to follow a diet based on The American Heart Association, National Cholesterol Education Program,[24] and received counselling on life-style modifications. Based on drug–drug interaction studies, there were no medications which could not be co-administered with dalcetrapib. However, patients were not allowed to take fibrates, bile acid sequestrants, niacin, rimonabant, or any medication (other than statins and lipid-lowering drugs) which would increase HDL-C levels. Care was taken not to change antihypertensive medication.

Predefined endpoints

The primary outcome parameters were: (i) the change from baseline in endothelial function in response to dalcetrapib over 12 weeks as assessed by FMD of the brachial artery using high resolution ultrasound and (ii) the change in 24 h ambulatory blood pressure monitoring (ABPM) at 4 weeks. The secondary outcome parameters were the effects of dalcetrapib on FMD at 36 weeks, the change in ABPM at 12 and 36 weeks, and of the following biomarkers: high-sensitive (hs) C-reactive protein; interleukin-6 (IL-6); soluble P-selectin (sP-selectin); soluble E-selectin (sE-selectin); soluble intercellular adhesion molecule-1; soluble vascular cell adhesion molecule-1; matrix metalloprotease-3; matrix metalloprotease-9; adiponectin, biomarkers of oxidation [myeloperoxidase (MPO)], and coagulation [tissue plasminogen activator (t-PA); plasminogen activator inhibitor-1 (PAI-1)]. Further, blood lipids, lipoproteins and apolipoproteins, CETP mass and activity, lipoprotein-phospholipase A_2 (LP-PLA_2) mass, insulin sensitivity, sodium, and potassium were determined. Clinical events recorded during the study were also assessed.

Flow-mediated dilatation

Brachial artery diameter was measured in the right brachial artery 5–10 cm proximal to the antecubital fossa at baseline and after 1 min of forearm occlusion (in %) with a high-resolution ultrasound probe (Ultrasonix SP ultrasound system and an L14, 10 MHz linear array broadband transducer).[25,26] An especially designed arm-rest and probe holder was constructed to optimize standardization of the position of the ultrasound probe. Measurements were obtained during 1 min, after which the blood pressure cuff was inflated to 250 mmHg in order to interrupt blood supply. Following 5 min of forearm ischaemia, the cuff was released and a second measurement was obtained during the following 3 min.

Blood flow velocity measurements

During FMD measurement, blood flow velocity and heart rate were continuously monitored by pulsed-wave Doppler and displayed as a spectral Doppler curve. The velocity time integral (VTI) was measured (off-line) using custom-made, automated flow analysis software (Medical Imaging Applications, IA, USA). The VTI was calculated for each R-wave triggered cardiac cycle as a percent increase from baseline. Measurements were made three times at baseline (first 60 s of scan) and during reactive hyperaemia (peak VTI following cuff release). The FMD flow stimulus during reactive hyperaemia was expressed as the ratio of peak to baseline volume-flow per minute.

Core laboratories

Flow-mediated dilatation was assessed by a central core laboratory (London Core Lab, London, UK). Sonographers were trained at two centralized training sessions by highly experienced teachers and certified by a pre-specified programme developed by the Core Lab. Training and study scans were read blindly by expert investigators using a computer-assisted analysis programme.

Blood chemistry, haematology, and coagulation parameters were measured in a core laboratory (Quintiles Laboratories Europe, The Alba Campus, Rosebank, Livingston, EH54 7EG, UK) as were lipid levels, cytokines, and other biomarkers (MedPace Reference Lab Europe, Technologielaan 19, 3100 Leuven, Belgium).

Ambulatory blood pressure monitoring recordings were analysed in a core laboratory (Spacelabs Healthcare, Rosanne House, Parkway, Welwyn Garden City, Herts, AL8 6JE, UK).

Data monitoring

Imaging data were stored in a central database (Amsterdam Medical Center, Amsterdam, NL, USA), while the clinical database was with Quintiles Inc. (Global Headquarters, Durham, NC, USA).

Statistical plan and analysis

The study was designed to test two primary hypotheses:[21] that dalcetrapib is non-inferior to placebo with regard to (i) endothelial function (by FMD) and (ii) changes in 24 h ABPM. The change from baseline to 12 weeks in brachial artery %FMD was compared between treatment groups using the intention-to-treat principle and included all patients randomized with a post-baseline efficacy assessment at 12 weeks. Patients were assigned to treatment groups as randomized. The pre-specified boundary for non-inferiority in %FMD of -0.65% for the difference (dalcetrapib minus placebo) was based on the assumptions that the average baseline %FMD would be approximately 4–4.5% and that a $>15\%$ relative decrease in FMD in the dalcetrapib arm after 12 weeks of treatment would indicate a disadvantageous effect on endothelial function. Based on previous assessments of reproducibility with a standard deviation of %FMD change from baseline of 2%, 200 patients in each treatment group were considered sufficient to provide 90% power to establish non-inferiority, assuming a two-sided α of 0.05. To account for a 10% dropout in follow-up, it was planned to randomize 450 patients. In the event that non-inferiority was demonstrated, a test for superiority using a two-sided significance level of 5% was planned. The change in %FMD from baseline to 36 weeks compared between treatment groups was a secondary outcome parameter. Other secondary outcome parameters included the effects of 36 weeks' dalcetrapib treatment on blood lipids, CETP activity, and biomarkers of inflammation and oxidation. No corrections for multiple comparisons were made.

The other primary outcome parameter was the change in ABPM from baseline between treatment groups after 4 weeks' and all patients who received at least one dose of study medication were included. The statistical plan pre-specified that analysis was as randomized,[21] however, in the event of patients switching from their initially randomized treatment, they could be assigned to treatment groups as an as-treated population for analysis purposes. The non-inferiority margin for the change from baseline (dalcetrapib−placebo) was +2 mmHg. Assuming a standard deviation of 6 mmHg for the 24 h ABPM change from baseline, 200 patients in each treatment arm were considered to be sufficient to establish non-inferiority with 90% power using a two-sided α of 5%. Non-inferiority was to be concluded if the upper limit of the 95% confidence interval (CI) for the difference (dalcetrapib−placebo) in change from baseline did not exceed +2 mmHg. Allowing for up to 10% dropouts, enrolment of 450 patients was considered sufficient. Secondary safety endpoints included change from baseline in mean ABPM after 12 and 36 weeks, adverse events, laboratory parameters, ECG, and vital signs. Cardiovascular events were recorded and adjudicated by the Clinical Events Committee (CEC).

For the primary and secondary efficacy and safety analyses, a linear model was assumed, including treatment and centre (small centres were grouped together) as fixed effects along with a covariate term for the baseline value of %FMD or 24 h ABPM. Results are reported in terms of estimated effect sizes, 95% CIs, and *P*-values. Baseline characteristics of the intent-to-treat population were compared between study groups with the use of descriptive statistics.

Between-group differences in %FMD and ABPM were also examined in pre-specified subgroups: HDL-C$\leq$40 mg/dL, HDL-C $>$ 40 mg/dL, LDL-C$\leq$80 mg/dL, LDL-C $>$ 80 mg/dL, hypertension, diabetes, and current smoking. In addition, to confirm the robustness of the primary analyses, key analyses of FMD and ABPM were repeated based on imputing missing values using the last observation carried forward method.

Role of the sponsor

The sponsor participated in discussions regarding the design and conduct of the study with the steering committee members and provided logistical support. Data and their analysis were assessed jointly. T.F.L. developed the initial draft of the manuscript with input from J.J.P.K. and J.E.D., and all authors were involved in reviewing and revising the manuscript prior to submission. All members of the steering committee had full access to the data and final responsibility for the decision to submit for publication.

Results

Patient characteristics and patient flow

We screened 860 patients from 19 centres. Of which, 476 were randomized and 466 participated in the trial (*Figure 1*). Four patients did not receive treatment and 10 were excluded due to lack of evaluable efficacy data. This left 472 and 466 patients to be considered for the safety and intention-to-treat primary analyses, respectively. The study arms were well balanced with regards to patient characteristics (*Table 1*). As primarily subjects with average or low HDL-C were recruited, males were predominant in this patient population.

Flow-mediated dilatation

Baseline FMD was 4.1 $\pm$ 2.2 and 4.0 $\pm$ 2.4% in the placebo and dalcetrapib groups, respectively, and did not change with dalcetrapib

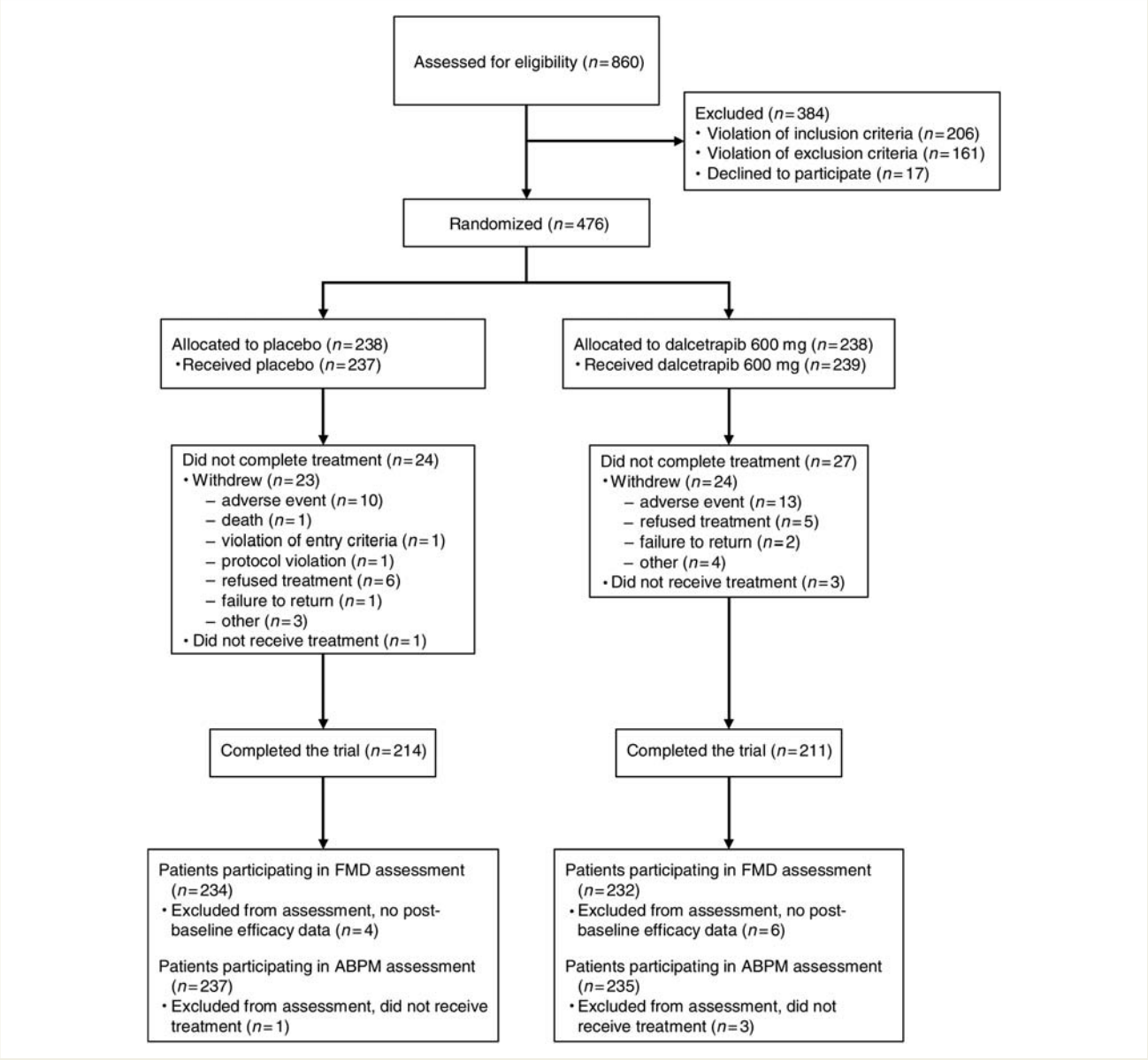

Figure 1 Screening, randomization, and follow-up of study subjects.

for up to 36 weeks (*Figure 2*). At week 12, the placebo-corrected change from baseline was −0.23 (−0.55, 0.10 95% CI; $P = 0.1764$), and the primary endpoint met the pre-specified non-inferiority criteria. At week 36, the corresponding value was −0.01 (−0.46, 0.43; $P = 0.9516$). Similarly, FMD did not differ between predefined subgroups, i.e. patients with low or high HDL-C, diabetics, hypertensives, or younger (<62 years) and older patients (>62 years; Supplementary material online, *Table S1*).

Blood flow velocity

At baseline, hyperaemia (i.e. VTI_p/baseline VTI_b) was assessed in 198 patients on placebo and 197 on dalcetrapib. VTIp/VTb was 511 ± 201% in the placebo group and 521 ± 193% in the dalcetrapib group. At week 12, the corresponding values were 525 ± 411 and 523 ± 195% ($P = 0.7383$ for placebo-corrected change from baseline) and at 36 weeks 540 ± 206 and 524 ± 201% ($P = 0.4381$) in the placebo and dalcetrapib groups, respectively (Supplementary material online, *Table S2*).

Blood pressure

Ambulatory blood pressure monitoring at weeks 4, 12, and 36 was unchanged (*Figure 3* and Supplementary material online, *Table S3*). At baseline, ABPM was 125.2 ± 11.7/74.2 ± 8.2 mmHg for the placebo and 127.6 ± 11.2/74.9 ± 7.2 mmHg for the dalcetrapib group. At week 4, the placebo-corrected change from baseline was 0.65 (95% CI, −0.68, 1.99; $P = 0.3372$) for systolic and 0.64 (−0.18, 1.45; $P = 0.1248$) for diastolic BP, and met the

Table 1 Summary of baseline clinical characteristics

Characteristic[a]	Placebo ($n = 234$)	Dalcetrapib ($n = 232$)
Age, years	61.9 ± 7.92	62.3 ± 7.05
Male sex, *n* (%)	211 (90)	211 (91)
Body-mass index	28.7 ± 4.4	29.6 ± 4.8
Medical history, *n* (%)		
Coronary heart disease	155 (66)	147 (63)
Symptomatic carotid artery disease	18 (8)	16 (7)
Peripheral arterial disease	16 (7)	24 (10)
Abdominal aortic aneurysm	5 (2)	6 (3)
Type II diabetes	102 (44)	108 (47)
Hypertension	175 (75)	171 (74)
Smoker, *n* (%)		
Ever	191 (82)	181 (78)
Current	57 (24)	65 (28)
Previous or concomitant treatment[b]		
Statin, *n* (%)	228 (97)	223 (94)
Angiotensin converting enzyme inhibitor, *n* (%)	86 (36)	89 (38)
Angiotensin receptor antagonist, *n* (%)	65 (28)	68 (29)
Salicylates[c], *n* (%)	147 (62)	154 (65)
Calcium channel antagonist, *n* (%)	70 (30)	66 (28)
Systolic BP, mmHg[d]	134.6 ± 14.95	135.5 ± 13.58
Diastolic BP, mmHg[d]	78.9 ± 9.45	80.1 ± 7.92
Plasma lipid profile		
HDL-C, mmol/L	0.995 ± 0.185	1.013 ± 0.190
apoA-I, g/L	1.333 ± 0.189	1.347 ± 0.178
LDL-C, mmol/L	2.051 ± 0.457	2.108 ± 0.553
apoB, g/L	0.874 ± 0.170	0.895 ± 0.185
Total cholesterol, mmol/L	3.802 ± 0.563	3.945 ± 0.665
Triglycerides, mmol/L	1.654 ± 0.733	1.819 ± 0.916

[a]All reported as mean ± SD unless otherwise stated.
[b]Patients with at least one treatment, multiple occurrences of the same treatment in one individual counted only once.
[c]Includes aspirin, carbasalate calcium, and aspirin DL-lysine.
[d]Measured using the auscultatory method.

pre-specified non-inferiority criteria for the randomized analysis. Throughout the trial, ABPM did not change significantly in the entire population or in predefined subgroups (low vs. high HDL-C, diabetics vs. non-diabetics, old vs. young). Notably, the percentage of 'non-dippers' (i.e. patients without night-time blood pressure decrease) was similar at baseline and increased with placebo, but decreased with dalcetrapib (Supplementary material online, *Table S2*).

Lipids

At baseline, HDL-C was 38.4 ± 7.1 and 39.1 ± 7.3 mg/dL in the placebo and dalcetrapib groups, respectively. Dalcetrapib increased placebo-corrected HDL-C by 25, 27, and 31% at weeks 4, 12, and 36, respectively (all $P < 0.0001$; to 49.7 ± 11.7, 49.2 ± 10.4 and 50.7 ± 12.7 mg/dL; *Figure 4*, Supplementary material online, *Table S4*). After 4, 24, and 36 weeks of treatment with dalcetrapib, CETP activity (placebo corrected) decreased by 50.9, 52.5, and 56.0%, respectively (all $P < 0.0001$). Placebo-corrected apolipoprotein A_1 levels increased with dalcetrapib by 9% at week 4 and 10% at weeks 24 and 36 (all $P < 0.0001$). Placebo-corrected triglyceride levels decreased by 9 and 14%, respectively ($P < 0.005$; from 161 ± 81 to 151 ± 83 and 149 ± 71 mg/dL, respectively; *Figure 4*). A small, but significant decrease in LDL-C of 4% ($P < 0.05$) was observed at week 4 only. Placebo-corrected apolipoprotein B100 decreased significantly by 4, 3, and 5% at weeks 4, 24, and 36 ($P < 0.05$; *Figure 4*).

Laboratory values

Plasma levels of sodium, potassium, and creatinine did not change significantly nor did the glucose:insulin ratio (data not shown). Although a placebo-corrected decrease in haemoglobin A_{1c} of 0.1% was observed at week 36 ($P < 0.05$), this was due to an increase with placebo with no change with dalcetrapib.

Markers of inflammation

Plasma levels of hs-C-reactive protein, ICAM-1, VCAM-1, IL-6, MPO, t-PA, or PAI-1 did not differ at baseline nor during treatment in both the placebo and dalcetrapib groups. Placebo-corrected Lp-PLA_2 mass increased by 17.4% (without correcting for HDL-C plasma levels; $P < 0.001$; Supplementary material online, *Table S5*).

Adverse clinical events

Adverse events were mild to moderate in intensity and included nasopharyngitis and influenza, diarrhoea, back pain, and headache with similar distribution among the treatment groups (*Table 2*). Twenty-three pre-specified positively adjudicated events occurred in 7 patients given dalcetrapib (11 events) and 8 patients given placebo (12 events); among them 7 major cardiac events (5 with placebo and 2 with dalcetrapib) and 16 revascularization procedures (7 in the placebo and 9 in the dalcetrapib group). No strokes were noted.

Discussion

The dal-VESSEL trial was undertaken to establish the safety and vascular efficacy of dalcetrapib in a patient population with CHD or a CHD equivalent risk profile. It used FMD as the primary outcome measure as the endothelium is the key signal transducer for vascular homeostasis and responds rapidly to both toxic and beneficial influences. Our findings show that, in contrast to torcetrapib,[11] dalcetrapib does not worsen endothelial function, nor does it raise BP as found with torcetrapib. This together with other safety data suggests that the large ongoing clinical programme investigating the effects of dalcetrapib on atherosclerotic plaque and CV outcomes should be continued.[27]

Since the early 1990s, statins have been shown to be highly effective in reducing major CV events in a series of large-scale clinical trials. Nevertheless, a considerable risk remains even after

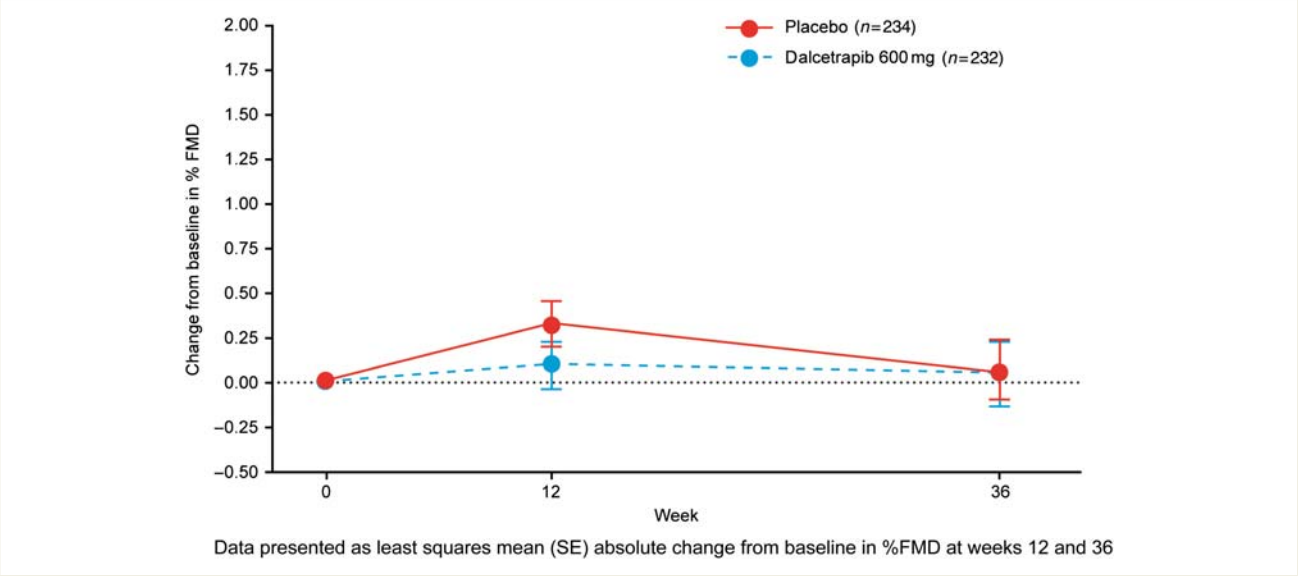

Figure 2 Flow-mediated dilatation (FMD) of the brachial artery in percent change from baseline as assessed by high resolution ultrasound of the brachial artery in patients on placebo (——; $n = 234$) or dalcetrapib (– –; $n = 232$). Data are mean ± SD.

achieving marked LDL-C reduction. In epidemiological studies, HDL-C levels are inversely related to CV risk.[1,28,29] Of note, this relationship is maintained even at very low LDL-C levels as achieved with the use of high-dose statin therapy.[4] These data, together with experimental work documenting beneficial effects of HDL-C on endothelial NO synthase expression and NO release,[12–16] the adhesion of white blood cells to the vessel wall,[30,31] and tissue factor expression,[32] has made HDL-C elevation an attractive target for drug therapy.

Infusion of reconstituted HDL in patients with hypercholesterolaemia improves endothelial function both in conduit arteries as well as in the microcirculation.[17] In patients with CAD, subcutaneous injection of apolipoprotein A_1 Milano over 5 weeks resulted in an increase in HDL-C and a reduced plaque volume as measured by intravascular ultrasound.[33] Since endothelial dysfunction precedes atherosclerosis,[34,35] these findings suggest that HDL-C exerts protective effects on the vasculature by activating the L-arginine/NO-pathway and in turn by reducing atherosclerotic plaque formation.

Recently, drugs which act as modulators or inhibitors of the CETP have been developed.[19,20,36,37] Cholesterol transport protein is a plasma protein that promotes the transfer of cholesterol esters from protective HDL to atherogenic LDL particles. Cholesterol transport protein inhibitors thereby produce large and consistent elevations of plasma HDL-C levels. However, the first compound of this type, torcetrapib, unexpectedly increased mortality in the large ILLUMINATE trial[7] in spite of marked increases in HDL-C and decreases in LDL-C plasma levels. Furthermore, in the RADIANCE-trial,[38] using B-mode carotid ultrasound as well as in the ILLUSTRATE-trial[39] using coronary intravascular ultrasound, torcetrapib did not reduce carotid intima-media thickness nor coronary plaque volume. Numerous studies have subsequently shown toxic 'off target' actions of torcetrapib, including elevations of plasma aldosterone,[8,11,18] vascular endothelin tissue levels,[9,11] and blood pressure[7] together with impaired endothelial function.[11]

The BP elevation observed with torcetrapib is not due to CETP inhibition itself, but is related to its specific molecular structure as other molecules of this class lack such effects.[8,18,40,41] Furthermore, CETP inhibitors have divergent pharmacological properties and potency on lipid subfractions. In the dal-VESSEL trial, using dalcetrapib we found an increase in HDL-C of 31%, which is less than that reported for torcetrapib (72%)[7] or anacetrapib (138%),[42] respectively. Low-density lipoprotein cholesterol did not change in contrast to torcetrapib and anacetrapib which lower LDL-C by 25 and 40%, respectively.[7,42] The increase in Lp-PLA_2 mass of 17% seen with dalcetrapib is likely related to the increase in HDL-C itself. These results suggest important pharmacological differences of these agents which may act as either inhibitors or modulators of CETP.[43]

Nevertheless, doubt has been cast on the rationale and safety of CETP inhibition as a therapeutic strategy. Thus, carefully designed, clinical programmes to determine the safety and efficacy of CETP inhibitors with the next generation of agents that target CETP are crucial. The dal-VESSEL trial is the first of a large portfolio of studies which are evaluating the impact of dalcetrapib on endothelial function and blood pressure (dal-VESSEL), plaque volume (dal-PLAQUE 1 and 2; clinicaltrials.gov number NCT00655473), and CV outcome (dal-OUTCOMES; clinicaltrials.gov number NCT00658515).[27]

In dal-VESSEL, endothelial function was chosen as a primary outcome, because the endothelium represents a key signal transducer linking circulating factors with the biology of the vessel wall and precedes and predicts atherosclerosis and clinical outcome.[44–47] It responds rapidly to beneficial and harmful stimuli and thus is expected to change in the presence of damaging

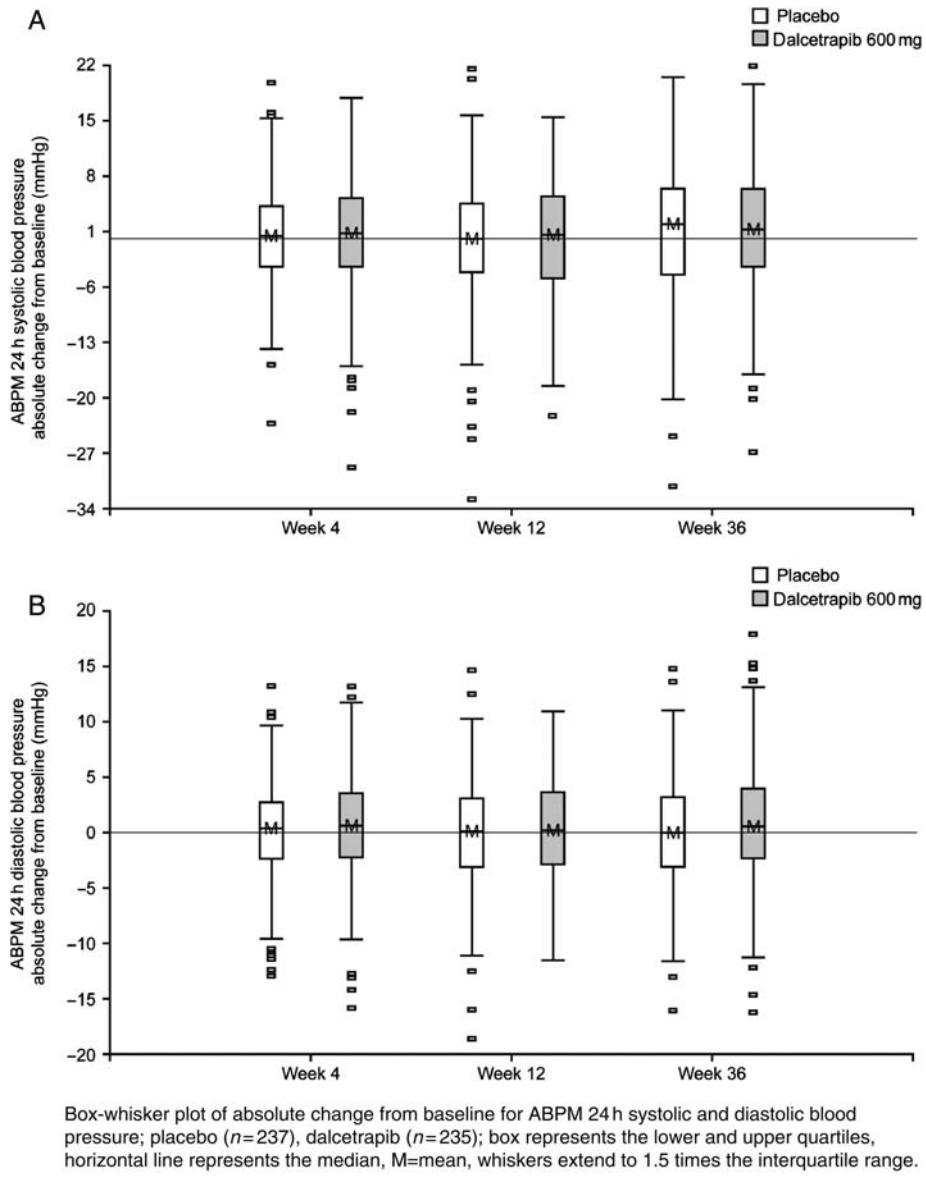

Figure 3 Change in 24 h ambulatory systolic (*A*) and diastolic (*B*) blood pressure after treatment with placebo ($n = 237$) or dalcetrapib ($n = 235$). Data are box-whisker plots $\pm$ 1.5 times the interquartile range.

effects secondary either to the molecule itself or to CETP inhibition. Flow-mediated dilatation has been shown to respond to pharmacological and dietary interventions. The changes in vascular reactivity to flow parallel other functions of the endothelium, including adhesion of white blood cells, inflammation, and coagulation.[48] The use of FMD in pharmaceutical trials has been limited by challenges of standardization of image acquisition and analysis as well as the innate biological variability of the measure itself. dal-VESSEL is the first multicentre trial to use FMD as a primary outcome measure. Flow-mediated dilatation is an appropriate endpoint as in a rodent model torcetrapib increased the vascular tissue levels of endothelin and reactive oxidant species, decreased eNOS expression and NO release, and impaired endothelium-dependent relaxation.[11] As expected in our high-risk patient population, baseline FMD was decreased in spite of optimal medical therapy according to the current guidelines. Although, based on previous studies, an improvement of FMD might have been expected, dal-VESSEL was designed as a non-inferiority trial, and while two co-primary endpoints were pre-specified no corrections for multiple comparisons were made. There was no evidence of impairment in endothelial function with dalcetrapib either at 12 or at 36 weeks, but we could also not confirm the beneficial effects of dalcetrapib reported in a small pilot study[22] or with reconstituted HDL[17] and niacin,[49] although with 476 patients enrolled dal-VESSEL would have been powered to show a possible superiority of dalcetrapib when compared with placebo on FMD as well. Drugs improving clinical outcome such as statins and ACE-inhibitors also improve FMD. However, there are exceptions

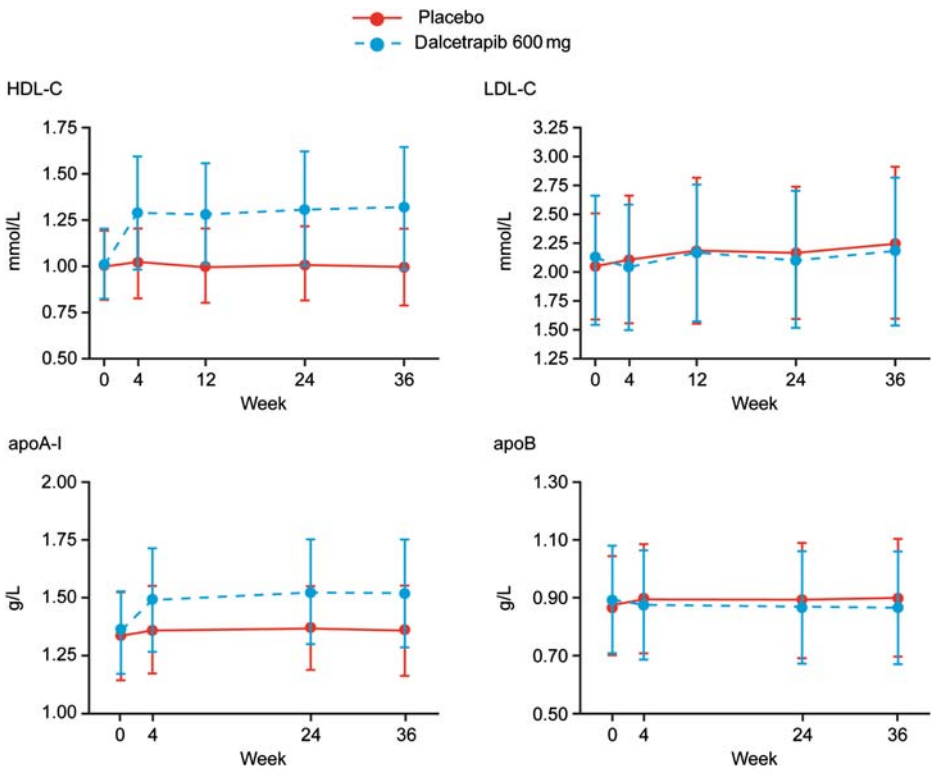

Figure 4 Changes in lipoprotein levels in patients treated with placebo (●——●) or dalcetrapib (●- - -●). Data are mean ± SD.

from this rule, such as estrogens[50] and vitamins[51,52], which partially limit the predictive value of FMD for the ongoing outcome trials.

It is unlikely that the neutral effects of dalcetrapib on FMD are due to methodological issues. Great efforts were made to train and certify sonographers involved in the 19 centres and to standardize the methodology for endothelial function testing. All sonographers underwent a structured training programme and were certified by a central core lab. Flow-mediated dilatation was performed according to a standardized protocol using especially constructed arm rests and probe holders. Furthermore, the study was overseen by a quality-control programme resulting in an unprecedentedly high level of reproducibility, equivalent to that seen in an expert laboratory. Ultrasound scans were read in a blinded fashion by highly experienced physicians in the core lab who were not involved in data acquisition.

It is possible that the increase in HDL-C with dalcetrapib was insufficient to improve FMD in this high-risk population as has been observed in a previous study.[22] Indeed, with intravenously applied reconstituted HDL, improvement of FMD was accompanied by much higher increases in HDL-C (75%).[17] Our patients had moderate impairment of FMD at baseline so that the measurements should have been able to detect both adverse and beneficial effects of treatment. In the dal-VESSEL trial, it is noteworthy that, despite a 30% increase in HDL-C, the neutral effects of dalcetrapib on FMD were paralleled by a lack of change in the circulating levels of markers of inflammation and oxidative stress such as MPO and hs-C-reactive protein. Interestingly, in the recent DEFINE study with anacetrapib, a much larger elevation in HDL-C was accompanied by no significant change in plasma C-reactive protein levels (from 1.4 to 1.5 mg/L). As in patients with CHD or other inflammatory conditions, HDL-C becomes dysfunctional and reduces rather than stimulates NO release, the inability of the current CETP inhibitors to suppress markers of inflammation needs further attention.[16,53]

In the ILLUMINATE trial, torcetrapib increased systolic blood pressure by about 5 mmHg,[7] an effect which is thought to have contributed to the observed increased mortality. In dal-VESSEL, ABPM at 4 weeks was therefore a primary outcome measure and was analysed blindly in a core laboratory. Importantly, neither systolic nor diastolic BP changed significantly with dalcetrapib confirming that dalcetrapib is different from torcetrapib and that hypertension is not a consequence of CETP inhibition itself.

The findings of the dal-VESSEL trial are reassuring in terms of the safety of this new agent. They support the continuation of research programmes studying agents that act on CETP, particularly to examine the longer-term effects of these agents on atherosclerosis plaque measures and clinical outcomes. dal-PLAQUE (clinicaltrials.gov number NCT00655473), and dal-OUTCOMES (clinicaltrials.gov number NCT00658515)[27] will be informative and indicate the impact of agents that act on CETP on both atherosclerosis evolution and CV events.

Table 2 Rates of adverse events and adjudicated cardiovascular events

Adverse event or safety variable	Placebo (n = 236)	Dalcetrapib 600 mg (n = 236)
Patients with at least one		
Adverse event	160 (68)	170 (72)
Total number of adverse events	483	437
Clinical adverse event leading to discontinuation of study drug	9 (4)	11 (5)
Serious adverse event	14 (6)	12 (5)
Drug-related serious adverse event	1 (<1)	2 (<1)
Adverse event leading to death	0	0
Most common adverse events (with incidence >5% of patients)		
Nasopharyngitis	42 (18)	38 (16)
Influenza	15 (6)	9 (4)
Diarrhoea	26 (11)	27 (11)
Back pain	16 (7)	8 (3)
Headache	13 (6)	10 (4)
Adjudicated cardiovascular events (CEC)[a]		
Death from coronary heart disease	1	0
Non-fatal myocardial infarction	3	2
Hospitalization for acute coronary syndrome[b]	1	0
Resuscitated cardiac arrest	0	0
Stroke	0	0
Subtotal composite (dal-OUTCOMES)[d]	5	2
Revascularization[c]	7	9
Total	12	11

[a]Clinical endpoints were adjudicated by an independent Clinical Endpoint Committee (CEC).
[b]One ECG abnormalities without biomarkers.
[c]Includes coronary and non-coronary.
[d]Subtotal composite including all-cause mortality, non-fatal MI, hospitalization for ACS (ECG abnormalities without biomarkers), resuscitated cardiac arrest and, fatal or nonfatal, stroke of presumed atherothrombotic aetiology.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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